1841-Pos Board B751
A Reinterpretation of Neutron Scattering Experiments on a Lipidated Ras Peptide Using Replica Exchange Molecular Dynamics
Scott Feller, Matthew A. Roark, Alexander Vogel.
The Ras family of proteins play crucial roles in a variety of cell signaling networks and mutations in these proteins are implicated in ~30% of human cancers. The various Ras proteins exhibit a high degree of homology in their soluble domains but extremely high variability in the membrane anchoring regions. We have employed replica exchange molecular dynamics computer simulations to study a doubly lipidated heptapeptide, corresponding to the C-terminus of the human N-Ras protein, incorporated into a dimyristoylphosphatidylcholine (DMPC) lipid bilayer. This same system has previously been investigated experimentally utilizing a number of techniques, including neutron scattering. Here we present results of well converged simulations that describe the subtle changes in scattering density in terms of the location of individual peptide sidechains and changes in lipid density arising from the inclusion of the lipid modifications to the peptide. The detailed picture that emerges from the combination of experimental and computational data exemplifies the power of combining isotopic substitution neutron scattering with high quality molecular dynamics simulation.

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Impact of Oxidized Lipids on Membrane Organization and Protein Misfolding
Marcus Wallgren, Lenka Beranova, Martin Hof, Nguyen Giang,
Gerhard Gröbner.
Oxidized phospholipids (oxPL) are now known to be involved in several pathological conditions, such as atherosclerosis, inflammation, infection, cancer, type 2 diabetes, and Alzheimer's disease. Further, phospholipid oxidation appears to be generically involved in apoptosis. However, a coherent overall view of the causalities and mechanisms is lacking, mainly because of insufficient understanding of the occurring processes on a cellular as well as a molecular level.
Here, we use a combination of Solid State NMR, Circular Dichroism and Calorimetry techniques to
i) to characterize the structural and dynamic organization of lipid bilayers at the presence of different oxLPs. Focus will be on their impact on membrane fatty acid order and dynamics in the membrane hydrophobic core, and occuring long range effects on the polar membrane region. First results show a severe impact of the presence of oxidized PC species on the phase behaviour of general PC and PC/PS vesicles. The size of the impact depends of the presence of an additional carboxyl or aldehyd group at the short sn-2 chain (ox. Product) and the length of these chain (5 versus nine carbons).
ii) The biophysical characterization of the impact of different oxLP species on the adsorption behaviour of peripheral proteins, in particular the amyloidogenic surface-active soluble Amyloid-β (Aβ) protein species and superoxide-dismutase 1 (SOD1) protein involved in Alzheimer's disease and Amyotrophic Lateral Sclerosis respectively. Which structural changes do these peptides undergo in the presence of these membranes? CD experiments have shown, that any conformational changes are a function of the type of oxidized lipids to be present in the used lipid model membranes.

1843-Pos Board B753
Dynamic Structure Formation of Membrane Proteins
Gernot Guigas, Diana Morozova, Matthias Weiss.
Cellular membranes are not mere passive envelopes but act as a reaction space for a multitude of vital cellular processes. While it is generally anticipated that biomembranes are highly dynamic and self-organizing entities, molecular mechanisms that underlie structure formation on lipid bilayers are still far from being fully understood. Here, we show by means of coarse-grained membrane simulations that proteins can form higher-order structures due to membrane-mediated interactions. Structure formation originates from characteristic protein-induced bilayer perturbations that particularly affect the coupling between membrane leaflets. Examining transmembrane proteins as well as peripheral membrane proteins, we observe the formation of protein oligomers and templates, even between proteins residing in different membrane leaflets. Also raft-like cross-leaflet associations of proteins and lipid patches are observed. Key parameter of this structure formation is the protein geometry. Apart from their potential influence on the organization of biomembranes, these effects may also support the formation of templates for signaling processes, the assembly of transport intermediates, or protein sorting events.

1844-Pos Board B754
***In Vitro* Studies on the Folding and Function of Lactose Permease in a Synthetic Lipid System**
Heather E. Findlay, Paula J. Booth.
Biological membranes are complex environments, where membrane proteins are surrounded by a bilayer composed of many different types of lipid. The physical properties of the bilayer influence protein structure, folding and function, while specific interactions with lipid molecules can also contribute towards the biological activity of some membrane proteins. Improving understanding of the interactions has resulted in the development of artifical lipid systems that allow the bilayer properties to be rationally manipulated *in vitro* to control protein behaviour. The bacterial transporter LacY is a well known integral membrane protein, responsible for the proton-driven uptake of D-lactose in *E. coli*. With a high resolution structure available and considerable understanding of mechanistic detail, and with observed changes to both structure and function in different bilayer environments, LacY is a good model system for examining the behaviour of a major class of membrane proteins in these lipid systems. Purified LacY has been folded and reconstituted into liposomes of varying synthetic lipid composition and the effect on protein topology and transport activity examined.

1845-Pos Board B755
Effects of Alpha Hemolysin from *E.coli* on Erythrocytes from Different Species
Susana A. Sanchez, **Romina Vazquez**, Sabina Maté, Laura Bakás,
Enrico Gratton, Vanesa Herlax.
Alpha-hemolysin (HlyA)is one of the key virulence factors released by *E. coli* strains. This toxin causes lysis of various mammalian cells, including erythrocytes of different animal species.
The hemolytic activity of the toxin on rabbit and sheep erythrocytes was determined, showing that rabbit is the most susceptible specie. Calcium concentration inside the erythrocytes while incubated with sublytic concentrations of HlyA was monitored using two-photon fluorescence Lifetime Imaging (FLIM), the calcium indicator Calcium Green 1 and the phasor analysis method. HlyA induces an increase in calcium concentration in both erythrocytes, but the increment in rabbit is 4 times higher and faster than in sheep. Two-photon Laurdan Generalized Polarization (GP) was used to determine the fluidity of the membrane (measured as the membrane water content) in the presence and absence of HlyA in live erythrocytes. The GP value for the sheep erythrocytes membranes was higher that the ones from rabbit and after incubation of the erythrocytes with sublytic concentration of Hlyn, an increase on the GP value was observed only on the rabbit membranes. Membrane lipid composition showed similar content of Cholesterol and PE in the two cell type, however the content of Phosphatidylcholine (PC) and sphingomyelin (SM) showed differences: sheep erythrocytes contained 28% of SM and not PC and Rabbit erythrocyte membranes present 10% SM and 18% of PC.
We concluded that at sublytical concentration, the initial interaction of HlyA with the erythrocyte and the mechanisms of calcium influx strongly depend on the membrane composition and fluidity of the target cell.
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Effect of SP-B and/OR SP-C on the Micro- and Nano-Structure of Synthetic Lipid Interfacial Films
Olga Lucia Ospina Ramirez, Luis Vázquez, Antonio Cruz, Jesus Perez-Gil.
Pulmonary surfactant is a lipid-protein complex which forms a continuous film at the lung air-liquid interface able to reduce surface tension stabilizing the respiratory surface against physical forces tending to collapse. Surfactant is composed of ~90% lipids, ~8-10% proteins, but less than 2% corresponds to hydrophobic proteins SP-B and SP-C. It has been reported that the lipid composition of surfactant leads to lateral phase segregation in bilayers and interfacial films, under physiologically-relevant conditions. The aim of this work was to analyze the effect of SP-B and/or SP-C on the micro- and nano-structure of films made of two surfactant-mimicking lipid mixtures. The mixture DPPC/POPC/POPG (50:25:15), with 0-5-10% of cholesterol, roughly mimics the lipid composition of natural surfactant. Alternatively, DPPC/POPG/Palmitate (68:22:9) mixtures are being widely used to produce clinical surfactant preparations. Monolayers of these two synthetic mixtures, with and without SP-B and/or SP-C purified from porcine lungs, have been transferred onto mica supports and analyzed by atomic force microscopy. All observed films exhibited coexistence of ordered and disordered phases, laterally segregated at